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Sleep Pharmacology of Typical and Atypical Ligands of Benzodiazepine Receptors

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GAILLARD, J.-M. AND R. BLOIS. *Sleep pharmacology of typical and atypical ligands of benzodiazepine receptors.* PHARMACOL BIOCHEM BEHAV **29**(4) 799–801, 1988.—The effects of several benzodiazepine and non-benzodiazepine ligands of benzodiazepine receptors have been investigated in sleep of normal young adults. The spectrum of activity of each compound has been characterized using a number of sleep variables in addition to the standard sleep stages. These substances affect all the principal components of sleep, that is the sleep-wake balance, paradoxical sleep, orthodox sleep and the EEG waveforms in the different sleep stages. Some, but not all, modifications induced by flunitrazepam are antagonized by flumazenil and they recover with various time constants after a single administration of the drug. The results of these experiments indicate a heterogeneity in the mechanism of action of benzodiazepine and non-benzodiazepine ligands of benzodiazepine receptors, because they affect differently the various components of sleep. It is not necessary to invoke a heterogeneity of the central benzodiazepine receptors (the BZ_1–BZ_2 theory) in order to account for these differences, but they can be explained by the concept of spare receptors.

Human sleep Benzodiazepines Non-benzodiazepine ligands Flunitrazepam Flumazenil Clonazepam Zopiclone Zolpidem PK 11195

THE effects of benzodiazepines (BZ) on sleep have been studied mostly from the point of view of their hypnogenic properties, whereas the more complex modifications they induce have attracted proportionally less attention. Their biochemical mode of action is fairly well understood, although some uncertainties remain, for instance the controversial issue of the existence of two different central receptors, the BZ_1 and BZ_2 types. However the application of this knowledge to understand their effects in man is not straightforward because of the complexity of the neural circuits involved in the expression of their clinical properties. This would require standard conditions in which precise measurements can be obtained. Sleep provides such a condition, and in the past few years we have studied several BZ and non-BZ ligands of BZ receptors in order to characterize more extensively their spectrum of activity on sleep in young normal adults [2,3].

In these experiments, healthy subjects have been recorded in the laboratory according to standard techniques. The laboratory sessions always started with one habituation night, not recorded, and consisted of several nights; however the various drugs were studied only in acute administration, and therefore were given only for one night. The subjects received placebo in the non-drug nights. The recordings were realized on magnetic tape and scored off-line with an automatic sleep stager developed 15 years ago in our laboratory [4]. This system gives, for each minute of the recording, the diagnosis of the sleep stage, along with numerical values for other variables, including the main EEG waveforms (delta, theta, alpha, κ potentials and spindles), rapid and slow eye movements, muscle tone and heart rate. The numerical results of each recording were averaged across all the subjects participating in a given experiment (n=8 to 20) and the difference between experimental conditions was statistically estimated using the Student *t*-test for paired samples, after estimation of the homoscedasticity of the variances with F-test. In addition, the general trends of sleep stages, describing their evolution as a function of sleep time, were calculated according to orthogonal polynomial fitting of the observed cumulated occurrences as previously described [1].

Flunitrazepam is a classical BZ with potent hypnogenic properties. It binds to the central and peripheral BZ receptors. However experiments with PK 11195, an antagonist of peripheral BZ receptors, have shown that the effects of flunitrazepam on sleep are related only to central BZ receptors, because they are not modified by combined administration with PK 11195.

Flunitrazepam modifies the sleep-wakefulness balance by increasing total sleep duration, by shortening sleep latency and by lengthening waking latency. This effect is visible only in the drug night, and disappears in the next night under placebo. It is fully antagonized by flumazenil, an antagonist of central BZ receptors. Interestingly, flumazenil has by itself a slight alerting effect: it increases the duration of wakefulness in the night, and delays sleep latency. These changes suggest an inverse agonistic effect (that is in a direction op-

posite to that of classical BZ), but observed only at relatively high doses (100 mg) and of smaller magnitude than the antagonistic effect.

Paradoxical sleep (PS) is affected by flunitrazepam in a way similar to wakefulness. The latency of PS is prolonged but the general trend shows that after this delay the production of the stage is parallel to that in the controls. This suggests that the drug exerts an all or nothing effect on this component of sleep, completely inhibiting it when the level of active substance is high enough, and allowing its normal production when this level falls below a threshold. However this is true for PS as a whole, but not for all of its components. Thus flunitrazepam decreases very potently the density of rapid eye movements in PS and both this effect and the inhibition of PS in the beginning of the night disappear in the placebo post-drug night. There is no clear indication for a rebound effect, because the general trend of PS is not significantly higher than in the controls. Flumazenil antagonizes the effect of flunitrazepam on PS, but not on the density of rapid eye movements. In addition the antagonist, when administered alone, has a slight agonistic effect and decreases the density of rapid eye movements in the second episode of PS of the night, presumably when its brain level is highest. Thus, this substance displays the puzzling property of having inverse agonistic effects on some sleep components (the sleep-wake balance), and agonistic effects on another one (rapid eye movements in PS).

Flunitrazepam modifies markedly orthodox sleep. Stage 2 is prolonged, an effect which persists in the placebo post-drug night, and is not antagonized by flumazenil. However if we consider two of the waveforms which characterize stage 2, a discrepancy becomes evident. Thus, flunitrazepam increases significantly the number of spindles and decreases the number of κ potentials. In the post-drug night the number of spindles returns to control values, whereas the number of κ potentials remains significantly depressed, showing a divergent evolution of these two components of stage 2. Moreover flumazenil completely prevents the flunitrazepam induced increase in spindles, but leaves the decrease of κ potentials unaffected.

The effect of BZ and non-BZ ligands of BZ receptors on stage 4 is more complex. Flunitrazepam increases stage 4 in the beginning of the night, for about two hours, and decreases it markedly in the rest of the night. Interestingly enough, this decrease becomes even more accentuated in the post-drug night, so that the total duration of stage 4 is shorter in that night under placebo than in the previous night under the effect of the drug. However other compounds induce different effects. Thus clonazepam, a BZ specific ligand of central BZ receptors, increases stage 4 for the full duration of the drug night [3]. A decrease is observed in the next placebo night. With zopiclone, a non-BZ ligand of central BZ receptors, the increase of stage 4 is more obvious with the dose of 7.5 mg than with 15 mg. Zolpidem, an imidazopyridine binding to central BZ receptors, increases stage 4 markedly and dose dependently. As with BZ, a decrease is observed in the post-drug night. Thus, it seems that BZ and other ligands exert two different effects on stage 4: a direct increase and an indirect decrease. With classical BZ like flunitrazepam, diazepam or bromazepam, the increase is weak and short-lasting. It is rapidly overcome by the secondary decrease, which may represent an adaptation reaction of the organism. With other compounds, either BZ like clonazepam or non-BZ like zopiclone and zolpidem, the increase is much more important and lasts for the entire drug night; the decrease appears only in the post-drug night.

The compounds causing predominantly a decrease of stage 4 are ligands of both central and peripheral BZ receptors, whereas the compounds increasing markedly stage 4 are ligands of central BZ receptors only. However it has been puzzling to observe that peripheral BZ receptors are not involved in this effect, since combination of flunitrazepam and PK 11195 affects stage 4 exactly as flunitrazepam alone. One is left with the conclusion that some other differences, perhaps in the way they bind to central receptors, must account for the differences in their effect on stage 4. This question is worth further investigations. Flumazenil decreases stage 4, an effect opposite to the primary effect of BZ, thus consistent with the possibility of small inverse agonistic properties of this drug.

Taken together, the effects of the different compounds briefly summarized above, the evolution of these effects comparing the drug and the placebo post-drug nights and the antagonism by flumazenil, indicate a heterogeneity in their mode of action. The components of sleep can be classified according to the way they react to these drugs. The sleep-wakefulness balance and PS are influenced by all compounds, an effect recovering relatively rapidly and antagonized by flumazenil, whereas the modification of orthodox sleep, particularly stage 2, is much longer lasting and not antagonized by flumazenil. An intermediate category is represented by rapid eye movements in PS, which are decreased by BZ, an effect no longer visible in the post-drug night but not antagonized by flumazenil. Finally, stage 4 constitutes a category by itself, due to the composite character of its modifications under BZ.

It is not necessary to invoke two different kinds of central BZ receptors to account for such a heterogeneity in the mode of action of BZ in man. A more parsimonious interpretation can be considered, suggested particularly by the fact that flumazenil antagonizes some effects of flunitrazepam, but does not antagonize other effects. It has been proposed by Haefely [5] that neurons in different systems contain different supply of the BZ receptor-GABA receptor-chloride channel complex, whereas the number of such complexes necessary to trigger a physiological effect is constant from one system to another. In other words, some systems have more spare receptors than others. It can be expected that the effect of a BZ on neurons containing few spare receptors is more transient and more easy to antagonize than on neurons containing a large number of spare receptors.

If this interpretation is true, it should be possible to classify the components of sleep according to the supply of BZ receptors-GABA receptor-chloride channel complexes on the neurons involved in their control. Accordingly, the neuronal systems responsible for sleep-wakefulness and PS regulation would contain a limited number of such complexes whereas those involved in orthodox sleep would contain a large number of them. In this way, sleep pharmacology can contribute to the investigation of physiological sleep mechanisms.

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